Increased Balloon-Induced Inflammation, Proliferation, and Neointima Formation in Apolipoprotein E (ApoE) Knockout Mice

Matter, Christian M ; Ma, Limming ; von Lukowicz, Tobias ; Meier, Patricia S ; Lohmann, Christine ; Zhang, Dongming ; Kilic, Ulkan ; Hofmann, Eugen ; Ha, Sook Wo ; Hersberger, Martin ; Hermann, Dirk M ; Luscher, Thomas F

Abstract: BACKGROUND AND PURPOSE:The pathophysiology of vascular lesions after balloon angioplasty remains poorly understood. A major limitation of most experimental studies in this regard is that injury was assessed in healthy arteries. Our aim was to study the effects of hypercholesterolemia in a mouse vascular injury model that mimics human balloon angioplasty. METHODS: Carotid balloon distension was performed in wild-type (WT) mice on a normal diet (ND), in apolipoprotein E-deficient (ApoE-/-) mice on ND and in ApoE-/- mice fed a high cholesterol diet (CD). RESULTS: Medial cell death (TUNEL) was elevated in all mice at 1 hour and 1 day after angioplasty without differences between the groups. We found enhanced intimal inflammation (%CD45-positive cells) and vascular cell adhesion molecule-1 expression at 7 days ($P < 0.05$; $n > \text{or} = 4$) as well as increased proliferation rates (BrdU-index) in ApoE-/- CD at 7 and 28 days postinjury ($P < 0.05$; $n > \text{or} = 5$). Four weeks after injury, these events led to enhanced neointima in ApoE-/- CD compared with WT ND mice (intima/media, $P < 0.001$; $n > \text{or} = 8$). The amount of lesion formation paralleled the incremental increase in total plasma cholesterol in WT ND, ApoE-/- ND and ApoE-/- CD ($P < 0.01$). CONCLUSIONS: Carotid balloon distension injury in ApoE-/- mice on CD induced enhanced inflammation and proliferation leading to increased neointima. Further applications of this microballoon catheter in genetically modified mice will provide opportunities to elucidate molecular mechanisms of vascular lesion formation in a model that reflects clinical balloon angioplasty. This know-how may pave the way to catheter-based interventions of human microvessels in the peripheral or cerebral circulation.

DOI: https://doi.org/10.1161/01.STR.0000241068.50156.82

Increased Balloon-Induced Inflammation, Proliferation, and Neointima Formation in Apolipoprotein E (*ApoE*) Knockout Mice

Christian M. Matter, MD; Liming Ma, MD; Tobias von Lukowicz, MD; Patricia Meier; Christine Lohmann; Dongming Zhang, MD; Ülkan Kilic, PhD; Eugen Hofmann; Suk-Woo Ha, PhD; Martin Hersberger, PhD; Dirk M. Hermann, MD; Thomas F. Lüscher, MD

Background and Purpose—The pathophysiology of vascular lesions after balloon angioplasty remains poorly understood. A major limitation of most experimental studies in this regard is that injury was assessed in healthy arteries. Our aim was to study the effects of hypercholesterolemia in a mouse vascular injury model that mimics human balloon angioplasty.

Methods—Carotid balloon distension was performed in wild-type (WT) mice on a normal diet (ND), in apolipoprotein E–deficient ($ApoE^{-/-}$) mice on ND and in $ApoE^{-/-}$ mice fed a high cholesterol diet (CD).

Results—Medial cell death (TUNEL) was elevated in all mice at 1 hour and 1 day after angioplasty without differences between the groups. We found enhanced intimal inflammation (%CD45-positive cells) and vascular cell adhesion molecule-1 expression at 7 days ($P<0.05$; $n\geq4$) as well as increased proliferation rates (BrdU-index) in $ApoE^{-/-}$ CD at 7 and 28 days postinjury ($P<0.05$; $n\geq5$). Four weeks after injury, these events led to enhanced neointima in $ApoE^{-/-}$ CD compared with WT ND mice (intima/media, $P<0.001$; $n\geq8$). The amount of lesion formation paralleled the incremental increase in total plasma cholesterol in WT ND, $ApoE^{-/-}$ ND and $ApoE^{-/-}$ CD ($P<0.01$).

Conclusions—Carotid balloon distension injury in $ApoE^{-/-}$ mice on CD induced enhanced inflammation and proliferation leading to increased neointima. Further applications of this microballoon catheter in genetically modified mice will provide opportunities to elucidate molecular mechanisms of vascular lesion formation in a model that reflects clinical balloon angioplasty. This know-how may pave the way to catheter-based interventions of human microvessels in the peripheral or cerebral circulation. (***Stroke*. 2006;37:2625-2632.)**

Key Words: cholesterol ■ angioplasty ■ inflammation ■ apolipoprotein E

Restenosis, the result of neointima formation and remodeling, still decreases the long-term clinical success of percutaneous coronary and peripheral interventions.[1] Local therapies such as drug-eluting stents have successfully reduced the rate of balloon- or stent-induced restenosis.[2] However, many of its molecular mechanisms remain poorly understood. Transgenic technologies in mice provide a powerful tool to address this problem.[3] Furthermore, apolipoprotein E knockout ($ApoE^{-/-}$) mice[4] with or without high cholesterol diet offer a model of marked hypercholesterolemia and atheroma reflecting the context of human atherosclerosis. Therefore, the challenge for vascular biologists in recent years was to establish a murine model of human restenosis. However, as balloon catheters amenable to mouse vessels were not available, the previous types of murine vascular injury such as wire injury,[5] fluid instillation and air desiccation,[6] spring[7] or cuff injury[8] at best tried to imitate balloon injury. Applying the concept of "response to injury",[9] it is likely that the *nature* of the injury determines the relative role of the following molecular and cellular events that ultimately lead to restenosis.

Therefore, we have designed a miniature balloon catheter which enabled us to characterize a murine balloon injury model that mimics balloon angioplasty in humans. By comparing the events after carotid injury between $ApoE^{-/-}$ mice on high cholesterol diet (CD) or normal diet (ND) and wild-type (WT) mice on ND, we demonstrate that $ApoE^{-/-}$ mice exhibit enhanced inflammation and proliferation leading to increased neointima formation.

Received June 24, 2006; final revision received July 21, 2006; accepted August 4, 2006.

From the Cardiovascular Research (C.M.M., L.M., T.v.L., P.M., C.L., D.Z., T.F.L.), Institute of Physiology, Zurich University and Cardiology, CardioVascular Center, University Hospital Zurich; the Center for Integrative Human Physiology (C.M.M., T.v.L., P.M., C.L., Ü.K., M.H., D.M.H., T.F.L.), Zurich University; the Department of Neurology (Ü.K., D.M.H.), University Hospital Zurich; Schneider Europe (E.H.), Bulach; Jomed (S.-W.H.), Beringen; Institute of Clinical Chemistry (M.H.), University Hospital Zurich, Switzerland.

Correspondence to Christian M. Matter, MD, Cardiovascular Research, Institute of Physiology, Zurich University and Cardiology CardioVascular Center, University Hospital Zurich, Winterthurerstrasse 190, CH-8057 Zurich, Switzerland. E-mail cmatter@physiol.unizh.ch

***Stroke* is available at http://www.strokeaha.org** **DOI: 10.1161/01.STR.0000241068.50156.82**

Materials and Methods

Animals and Diets

Male *ApoE*$^{-/-}$ (C57BL/6J) mice and their corresponding WT background strain were obtained from Jackson Laboratory (Bar Harbor, ME) and kept on a regular diet. An additional group of *ApoE*$^{-/-}$ mice was fed a CD (D12108 containing 1.25% cholesterol, Research Diets, New Brunswick, NJ) that was started 10 days before intervention and continued until harvesting. All animal experiments were performed in accordance with institutional guidelines and approved by the local animal committee.

Mouse Carotid Balloon Injury

WT and *ApoE*$^{-/-}$ mice (8 weeks old, around 25 g) were used for experiments. Balloon injury of the left proximal common carotid artery was performed by adapting a previously published protocol.[10] Briefly, animals were anesthetized injecting ketamine (75 to 95 mg/kg; Intervet; Zurich, Switzerland) and xylazine (4 to 8 mg/kg; Rompun, Bayer; Leverkusen, Germany) intraperitoneally. After dissecting the left carotid bifurcation, we ligated the external carotid artery distally, placed clamps on the internal and mid-common carotid artery and introduced the balloon catheter through an arteriotomy on the proximal external carotid artery (Figure 1). After

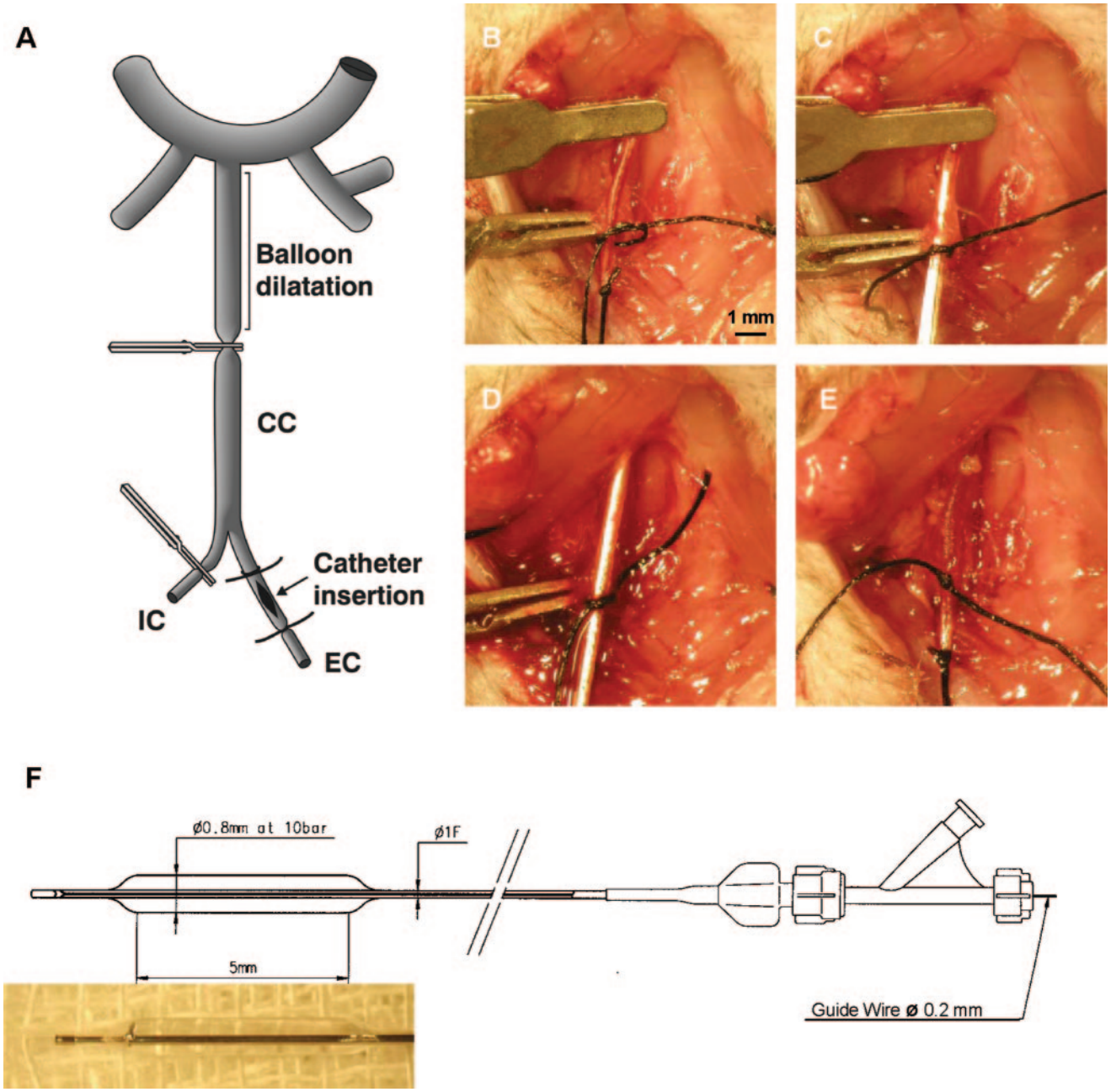

Figure 1. Carotid balloon distension in the mouse. Scheme depicting the surgical approach to balloon dilatation of the left proximal common carotid artery in the mouse (panle A). After exposing the left carotid bifurcation, the distal external carotid artery (EC) is ligated (panel B). The transient interruption of blood flow by clipping the internal (IC) and mid-common carotid artery (CC) allows insertion of the balloon catheter through an incision in the proximal EC (panel C). After dilating the proximal, nondissected (nonvisible) CC (panel D), the catheter is withdrawn and the clip on the mid-CC put back in place. After ligating the proximal EC and removing the clips, blood flow through CC and IC is reestablished (panel E). The balloon catheter is stiffened by a guide wire (panel F); the balloon length is 5 mm.

removing the clamp on the common carotid artery, the catheter was advanced to the proximal, nondissected common carotid artery where the balloon was distended for 40 seconds. Balloon size was matched to the weight of the animals using a balloon diameter of 0.77 mm for a 24-g mouse, 0.79 mm for a 25-g, and 0.80 mm for a 26-g mouse. The balloon catheter was specifically manufactured (Schneider, Bulach; then Jomed, Beringen, then Schwager Medica, Winterthur, Switzerland) with a balloon-length of 5 mm and a diameter ranging from 0.72 mm at 8 bars to 0.83 mm at 16 bars. The balloon was stiffened by a guide wire (0.2 mm diameter) and expanded using a water-filled inflation device (Monarch 25; Merit Medical).

Tissue Harvesting and Processing

Mice were euthanized 1 hour, 1 day , 7 days and 28 days after balloon distension injury. For the 2 latter time points, animals were injected with BrdU (50 mg/kg, Sigma) intraperitoneally 17 hours, 9 hours and 1 hour before harvesting as described.[11] After puncturing the left ventricle and cutting the right atrium, vessels were rinsed with phosphate-buffered saline (PBS) and perfusion-fixed at 100 mm Hg for 8 minutes using 4% paraformaldehyde (Sigma) in PBS. The injured left and the untouched right common carotid artery were excised after dissecting the adventitia. After postfixation with 4% paraformaldehyde for 2 hours and immersion in 30% sucrose overnight, vessels were embedded in optimal cutting temperature compound (Tissue-Tek), frozen, and stored at −80°C. For immunohistochemical analyses, vessels were rinsed with normal saline, embedded in optimal cutting temperature compound and frozen without fixation. For scanning electron microscopy, vessels were harvested 1 hour after balloon injury and perfusion fixation was carried out as above using glutaraldehyde 2% buffered with 0.1 mol/L cacodylate (Sigma) for 4 minutes. Vessels were opened longitudinally, dehydrated through series of ethanol, critical point-dried, spattered with 15 nm gold and examined in a scanning electron microscope 505 (Philips).

Morphology

Three serial cross-sections (5-μm thickness, 300-μm apart) were taken from the mid-portion of the dilated segment for histomorphological analysis (microscope BX51, Olympus). Corresponding sections were obtained from the untouched right common carotid artery as a negative control. Intimal and medial cell numbers were assessed using 4′-6-diamidino-2-phenylindole (DAPI; 5 μmol/L in PBS; Boehringer). Cell death was determined by TdT-mediated dUTP nick end-labeling (TUNEL; Roche). Cellular proliferation was characterized by staining with a biotin-conjugated mouse anti-BrdU antibody (Zymed, San Francisco, CA) and fluorescein-avidin DCS (1:200; Vector). Proliferation indices (BrdU-I) were calculated as percentage of total cell number. Endothelial cells were characterized by staining with a rat antimouse CD31 (557355, 1:2000; BD Pharmingen, Allschwil, Switzerland), vascular smooth-muscle cells by a biotinylated antirabbit smooth-muscle α-actin antibody (PK-

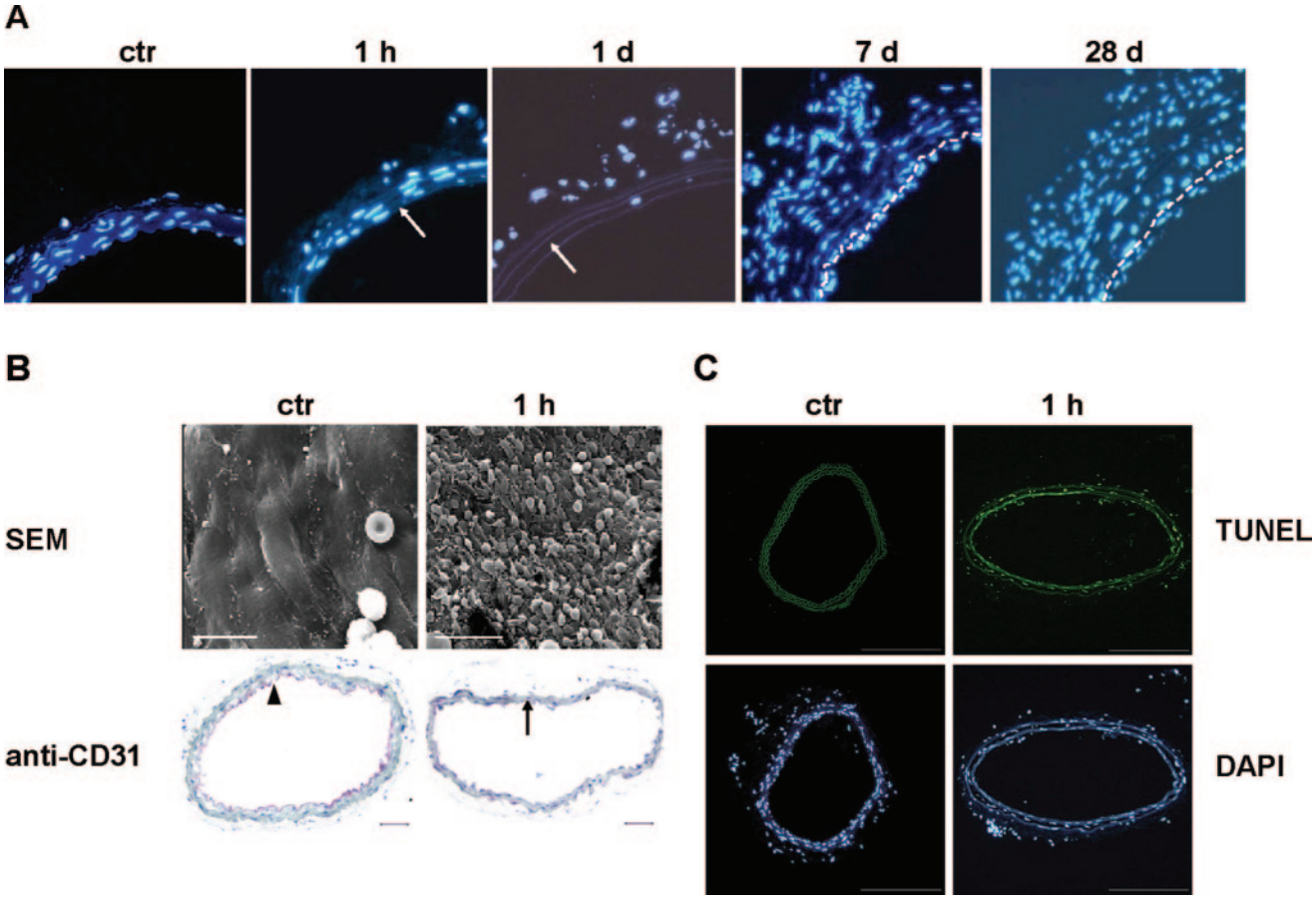

Figure 2. Stages of balloon-induced lesion formation (WT mice). A, Cross-sections of common carotid arteries without injury (ctr) and 1 hour (note loss of endothelial cells, *arrow*), 1 day (note loss of medial cells, *arrow*), 7 days and 28 days (*dashed line* indicating neointima-media border) after balloon distension injury in WT mice; chromatin staining using DAPI. B, Scanning electron microscopy (SEM) of untouched carotid artery (ctr) with intact endothelial layer and injured vessel 1 hour postballoon angioplasty (1 hour) with endothelial denudation and platelet monolayer; bar=10 μm. Anti-CD31 staining of cross-sections shows intact endothelial cells (*arrowhead*) in an untouched vessel and endothelial denudation (*arrow*) 1 hour after injury; bar=100 μm. C, Characterization of medial vascular smooth-muscle cell death using TUNEL staining reveals no positive cells in intact arteries (ctr), but many TUNEL-positive medial nuclei 1 hour after balloon injury. The index of cell death is illustrated by DAPI staining of the same samples; n=3, bar=200 μm.

6101, 1:200; Vector). Inflammatory cells were identified using a biotin-conjugated rat antimouse CD45 antibody (553077, 1:50; BD Pharmingen). Vascular cell adhesion molecule (VCAM)-1 expression was determined using a rat antimouse antibody (MCA1229, 1:200; Serotec). Morphometric analyses of areas were performed on sections with nuclear staining (DAPI) with the addition of polarized light; sections were photomicrographed (Olympus DP50-CU camera), digitized and analyzed (Analysis 5, SoftImaging System).

Statistical Analysis

All data are expressed as mean±SEM. Statistical significance of differences was calculated using ANOVA with post hoc Tukey test or Student unpaired *t* test. $P<0.05$ was considered statistically significant.

Results

Carotid Balloon Distension Injury in Mice Reflects Events After Balloon Angioplasty in Humans

Using a custom-made miniaturized balloon catheter, we performed local over-distension of the nondissected proximal portion of the left common carotid artery (Figure 1). Analyses of cross-sections of the dilated carotid arteries in WT mice showed that balloon distension induced an immediate endothelial cell loss after 1 hour, a marked decrease in medial cells at day 1, followed by cellular infiltrations at day 7 leading to neointima formation at day 28 (Figure 2A).

Our next step was to detail the early events after carotid balloon dilatation in WT mice. Microscopical analyses of carotid arteries 1 hour after balloon angioplasty revealed a platelet monolayer (Figure 2B, top), endothelial denudation (Figure 2B, bottom), as well as increased medial and adventitial cell death (Figure 2C).

Increased Neointima in *ApoE*$^{-/-}$ Mice Fed a CD

Hypercholesterolemia is a prominent risk factor leading patients to percutaneous catheter interventions. Therefore, we applied our balloon injury model in the following 3 groups: WT mice on a ND, *ApoE*$^{-/-}$ mice kept on a ND and *ApoE*$^{-/-}$ mice fed a CD.

Analyses of cross-sections 28 days after injury revealed moderate neointima formation in WT mice that was progressively increased in *ApoE*$^{-/-}$ on ND and *ApoE*$^{-/-}$ on CD (Figures 3A and 4A and B); neointima formation in injured arteries was increased in all 3 groups as compared with untouched vessels ($P<0.05$). At this time point, endothelial coverage was complete in all 3 groups (Figure 3B). Lumen narrowing was more pronounced in *ApoE*$^{-/-}$ on CD, but did not reach statistical significance. Furthermore, there was no difference in perimeters of external elastic laminae between the groups. However, distension injury induced some enlargement remodeling after 28 days as compared with untouched control arteries (please see supplemental Figures I and II, available online at http://stroke.ahajournals.org). Neointima formation in the different groups paralleled total plasma cholesterol levels (Figure 4C); plasma triglycerides remained unchanged (Figure 4D).

As a next step, we quantified total medial (Figure 4E) and intimal cells (Figure 4F) on carotid cross-sections at different time points after balloon injury and compared them to uninjured control arteries. Measurements at 1 hour and 1 day after balloon injury confirmed endothelial denudation and revealed a more pronounced decline in total medial cells in *ApoE*$^{-/-}$ on CD than in WT mice on ND ($P<0.05$). As balloon distension induced mechanical disruption of medial cells, chromatin staining identified an increased number of medial nuclei 1 hour after injury as compared with uninjured vessels. By day 7, total medial and intimal cells increased in parallel without a significant difference between the groups. Twenty-eight days postangioplasty, counts of total intimal cells

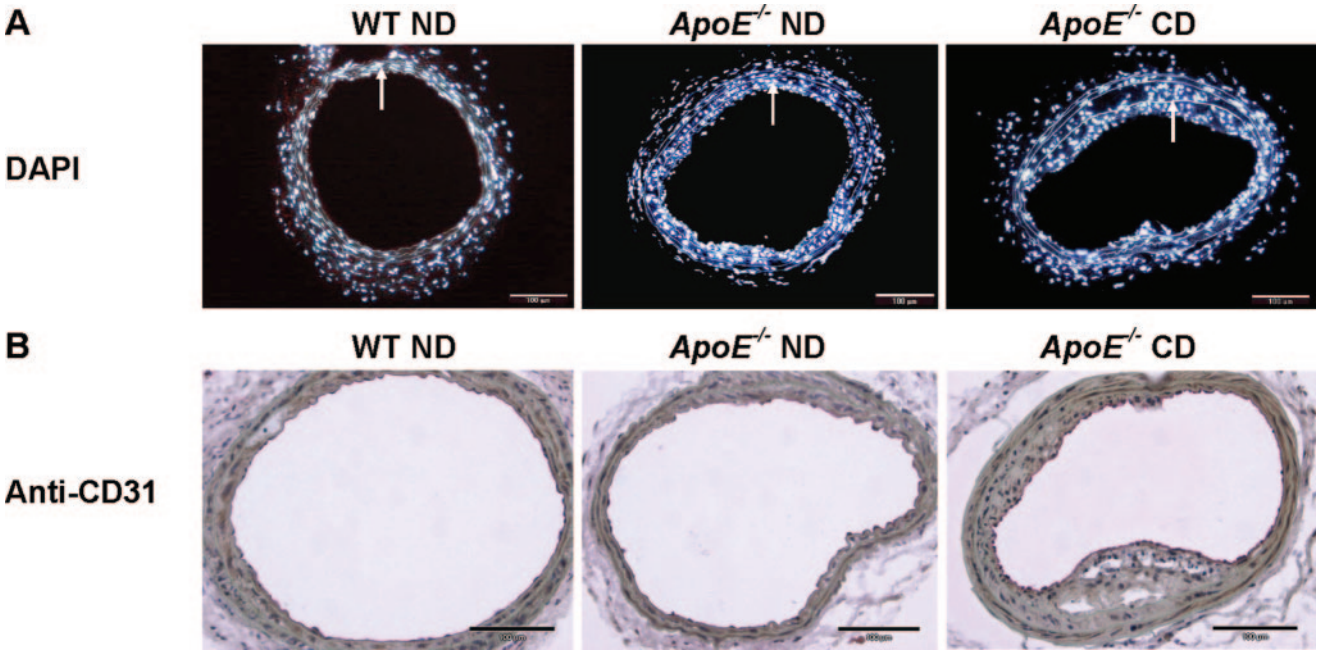

Figure 3. Balloon angioplasty induces neointima formation. A, Chromatin staining of cross-sections obtained 28 days postinjury from WT mice on ND (left), *ApoE*$^{-/-}$ on ND (middle) and *ApoE*$^{-/-}$ on CD (right). Arrows indicate the internal elastic lamina that delineates the neointima as its outer border. DAPI staining plus polarized light; bar=100 μm. B, Anti-CD31 staining identifies an intact endothelial layer in all 3 groups 28 days after balloon injury; representative samples of n=3, bar=100 μm.

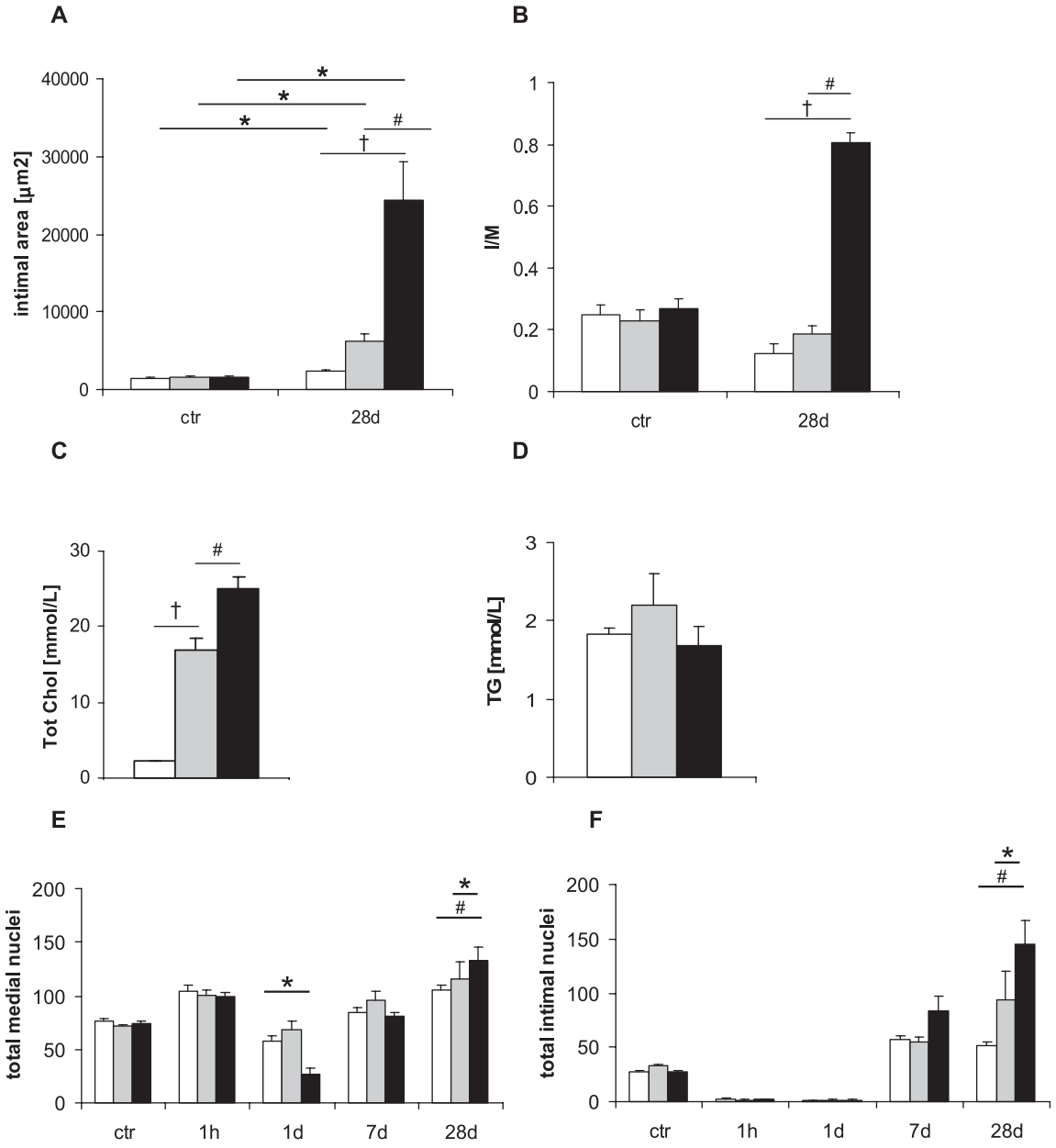

Figure 4. Increased neointima in *ApoE*$^{-/-}$ mice is associated with elevated cholesterol levels. A, Intimal areas and (B) intima/media ratio (I/M) 28 days postballoon angioplasty confirm increased neointimal lesions in *ApoE*$^{-/-}$ on CD (n=12, black columns) compared with *ApoE*$^{-/-}$ on ND (n=8, gray columns) and WT mice on ND (n=12, open columns); neointimal lesions are significantly different from untouched control vessels. C, Total plasma cholesterol levels are markedly elevated in *ApoE*$^{-/-}$ on CD and *ApoE*$^{-/-}$ on ND and parallel the amount of neointima formation (n=4). D, Triglycerides (TG) are unchanged between the groups. E and F, Analyses of total medial and intimal cells in untouched control vessels (ctr) and at 1 hour, 1 day, 7 days and 28 days after balloon injury (n≥8) reveal an early decrease in total medial cells at 1 day with recovery at later time points. Intimal cells virtually disappear up to 1 day after mechanical injury and progressively increase as a neointima thereafter. At 28 days postballoon injury, total intimal cell numbers are significantly increased in *ApoE*$^{-/-}$ on CD compared with WT animals. *P<0.05; #P<0.01; †P<0.001; ANOVA with post hoc Tukey test or Student unpaired *t* test, respectively.

were significantly higher in *ApoE*$^{-/-}$ mice on CD compared with *ApoE*$^{-/-}$ (P<0.05) or WT mice on ND (P<0.01).

Increased Proliferation and Inflammation in *ApoE*$^{-/-}$ on CD Compared With WT Mice

In order to get more insight into the kinetics of cell numbers after balloon injury, cell death and proliferation rates were analyzed. Cell death reached around 40% of medial cells at 1 hour postinjury and decreased later on; the differences between the groups were not statistically significant (please see supplemental Figures I and II). In contrast, medial and intimal proliferation rates at 7 and 28 days postinjury (Figure 5A and B) were significantly increased in both *ApoE*$^{-/-}$ on CD compared with *ApoE*$^{-/-}$ on ND as well as *ApoE*$^{-/-}$ on CD compared with WT mice (P<0.05). Neither cell death nor proliferation was detected in untouched control vessels.

Staining for smooth-muscle cells increased 7 days after balloon injury as compared with untouched arteries (P<0.05,

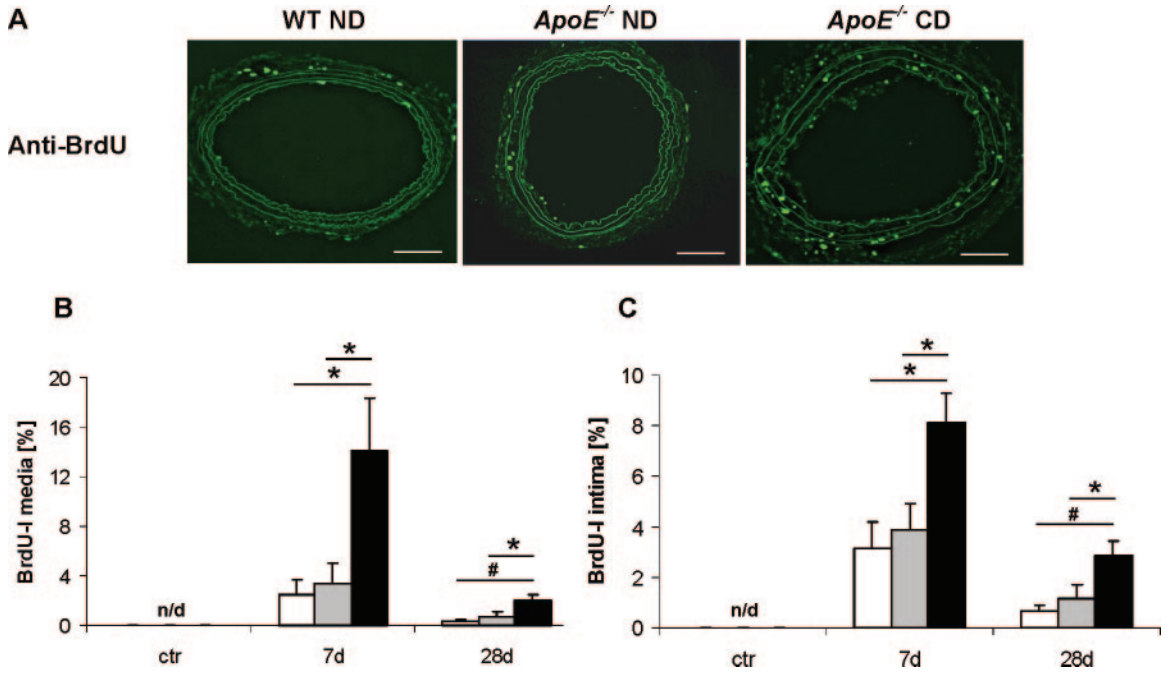

Figure 5. Increased proliferation in *ApoE*$^{-/-}$ mice on high cholesterol diet. A, Anti-BrdU stainings of carotid cross-sections 7 (A; n≥5) and 28 days (n≥6) after balloon injury reveal increased proliferation rates (BrdU-Index) in both (B) medial and (C) intimal cells; BrdU staining is not detectable (n/d) in untouched right common carotid arteries (ctr). WT ND (open columns); *ApoE*$^{-/-}$ ND (gray columns), *ApoE*$^{-/-}$ CD (black columns); *P<0.05; #P<0.01; ANOVA with post hoc Tukey test; bar=100 μm.

n=3), but injured vessels showed no difference between the groups (Figure 6A). In contrast, intimal leukocytes as well as intimal VCAM-1 expression were both significantly increased in *ApoE*$^{-/-}$ mice on CD as compared with WT mice (Figure 6B and 6C; P<0.05, n ≥4) and untouched control arteries (P<0.001; n=3). These results suggest additive effects of increased plasma cholesterol levels on inflammatory and proliferative responses after balloon injury.

Discussion

Using a mouse model of balloon injury, we demonstrate endothelial denudation, platelet activation, early medial cell death, followed by inflammation, proliferation, and neointima. Thus, our model reflects the stages of balloon- or stent-induced lesion formation known from human autopsy and atherectomy series.[12,13]

By comparing the effects of balloon injury between WT mice on ND, *ApoE*$^{-/-}$ on ND and *ApoE*$^{-/-}$ mice on CD, we have detailed the contribution of plasma cholesterol levels on lesion formation. Twenty-eight days after balloon injury, *ApoE*$^{-/-}$ mice on CD exhibited more pronounced neointima than *ApoE*$^{-/-}$ or WT mice on a ND. Enhanced vascular lesion formation in hypercholesterolemic *ApoE*$^{-/-}$ or *LDL-receptor* $^{-/-}$ mice has been described using a guide wire,[14] resin beads,[15] cuffs[16] or air desiccation combined with stretch.[17] However, most of these injury types have limitations with regard to their clinical relevance.

Medial cell death peaked very early after balloon injury and decreased quickly thereafter without significant difference between the groups. These findings match observations in larger rodents.[18] The elevated medial and intimal cell proliferation rate in *ApoE*$^{-/-}$ mice on CD suggests an oxidation-mediated stimulation of medial and intimal smooth-muscle cell proliferation leading to an increased neointima. These findings match data obtained in cell culture and rabbits showing enhanced smooth-muscle proliferation on oxidized low-density lipoprotein (oxLDL).[19] Interestingly, we identified the most significant increase in neointimal area and proliferation rates with the addition of dietary cholesterol in *ApoE*$^{-/-}$ mice. These morphological findings were associated with total plasma cholesterol levels in the different groups. In parallel, Plump and coworkers found a correlation of atherosclerotic lesion formation with total plasma cholesterol levels and addition of dietary cholesterol.[4]

Although cell death was not significantly different in the 3 groups examined, its early peak after balloon injury is likely to have implications on the following events. Indeed, dying cells may induce a positive feedback and promote—among other factors—the release of inflammatory cytokines.[16,20] In accordance with this notion, we observed enhanced recruitment of CD45-positive inflammatory cells and increased expression of VCAM-1 in *ApoE*$^{-/-}$ mice on CD 7 days after balloon injury. In addition, oxLDL may directly activate the coagulation cascade via activation of tissue factor in cultured smooth-muscle cells[21] or induce apoptosis in endothelial cells.[22] Thus, balloon angioplasty and oxLDL may induce a direct, combined injury on the vessel wall including endothelial denudation, cell death and activation of coagulation.[23] The ensuing "response to injury"[9] comprises an inflammatory reaction and proliferation.

We acknowledge that neointima formation in WT mice 28 days after balloon distension was moderate. This is likely attributable to an unopposed healing response in a healthy carotid artery. To increase the response to injury, we recom-

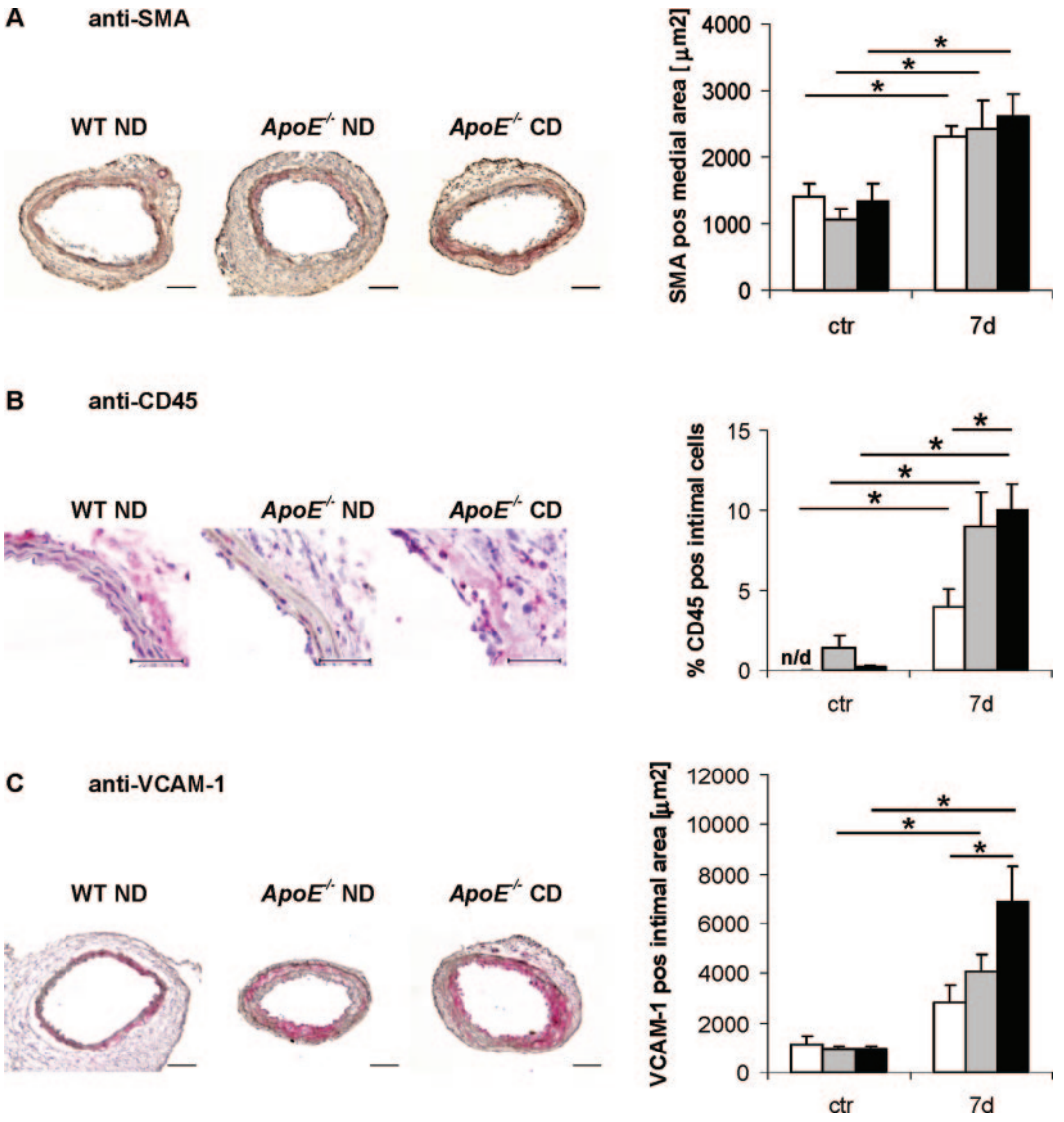

Figure 6. Increased vascular smooth-muscle cells and inflammatory response in *ApoE*$^{-/-}$ mice 7 days after carotid balloon injury. A, Immunohistochemical stainings of cross-sections show a progressive increase in anti–smooth-muscle α-actin (SMA) staining 7 days after balloon angioplasty as compared with untouched controls (ctr). Similarly, intimal inflammatory cells (B, anti-CD45 staining) and anti-VCAM-1 staining (C) reveal a markedly enhanced inflammatory response 7 days after injury compared with ctr; *$P<0.05$, ANOVA with post hoc Tukey test or Student unpaired *t* test, respectively; injured ($n\geq4$) versus ctr ($n=3$); bar=100 μm.

mend to (1) maximize balloon distension, (2) use aspirin via drinking water for decreasing vessel thrombosis, (3) increase plasma cholesterol levels (via CD or selection of an atherogenic genetic background for enhancing the response to injury, and (4) analyze neointima at 14 instead of 28 days.

In summary, we have characterized a novel mouse model of balloon distension injury which imitates the stages of vascular lesion formation induced by percutaneous transluminal angioplasty in the clinical context. *ApoE*$^{-/-}$ mice on a high cholesterol diet exhibit increased balloon-induced proliferation rates with enhanced neointima formation, in parallel to their elevated total plasma cholesterol levels. Further applications of this miniature balloon in arteries of genetically modified mice will provide attractive opportunities to elucidate molecular mechanisms of vascular injury such as apoptosis, inflammation or proliferation. This know-how may pave the way to catheter-based interventions of human microvessels in the peripheral or cerebral circulation.

Acknowledgments

We thank Peter Groscurth for helping us with scanning electron microscopy and Romeo Ricci for critical reading.

Sources of Funding

This work was funded in part by grants from the Swiss National Science Foundation (C.M.M., postdoc stipend) and 3100-068118 (T.F.L., C.M.M.), the Swiss Foundation for Stipends in Medicine and Biology (C.M.M.), Stiefel-Zangger Foundation (C.M.M.), the Swiss Heart Foundation (C.M.M.), Adumed Foundation (C.M.M.), the European Union G5RD-CT-2001-00532 and by Bundesamt für Bildung und Wissenschaft 02.0057 (C.M.M., T.F.L.) as well as support by Abbott/Jomed, Germany (C.M.M.).

Disclosures

None.

References

1. Mintz GS, Popma JJ, Pichard AD, Kent KM, Satler LF, Wong C, Hong MK, Kovach JA, Leon MB. Arterial remodeling after coronary angioplasty: a serial intravascular ultrasound study. *Circulation*. 1996;94:35–43.
2. Morice MC, Serruys PW, Sousa JE, Fajadet J, Ban Hayashi E, Perin M, Colombo A, Schuler G, Barragan P, Guagliumi G, Molnar F, Falotico R. A randomized comparison of a sirolimus-eluting stent with a standard stent for coronary revascularization. *N Engl J Med*. 2002;346:1773–1780.
3. Breslow JL. Mouse models of atherosclerosis. *Science*. 1996;272:685–688.
4. Plump AS, Smith JD, Hayek T, Aalto-Setala K, Walsh A, Verstuyft JG, Rubin EM, Breslow JL. Severe hypercholesterolemia and atherosclerosis in apolipoprotein E- deficient mice created by homologous recombination in ES cells. *Cell*. 1992;71:343–353.
5. Lindner V, Fingerle J, Reidy MA. Mouse model of arterial injury. *Circ Res*. 1993;73:792–796.
6. Simon DI, Dhen Z, Seifert P, Edelman ER, Ballantyne CM, Rogers C. Decreased neointimal formation in Mac-1(−/−) mice reveals a role for inflammation in vascular repair after angioplasty. *J Clin Invest*. 2000;105:293–300.
7. Sata M, Maejima Y, Adachi F, Fukino K, Saiura A, Sugiura S, Aoyagi T, Imai Y, Kurihara H, Kimura K, Omata M, Makuuchi M, Hirata Y, Nagai R. A mouse model of vascular injury that induces rapid onset of medial cell apoptosis followed by reproducible neointimal hyperplasia. *J Mol Cell Cardiol*. 2000;32:2097–2104.
8. Moroi M, Zhang L, Yasuda T, Virmani R, Gold HK, Fishman MC, Huang PL. Interaction of genetic deficiency of endothelial nitric oxide, gender, and pregnancy in vascular response to injury in mice. *J Clin Invest*. 1998;101:1225–1232.
9. Ross R. The pathogenesis of atherosclerosis: a perspective for the 1990s. *Nature*. 1993;362:801–809.
10. Napoli C, Aldini G, Wallace JL, de Nigris F, Maffei R, Abete P, Bonaduce D, Condorelli G, Rengo F, Sica V, D'Armiento FP, Mignogna C, de Rosa G, Condorelli M, Lerman LO, Ignarro LJ. Efficacy and age-related effects of nitric oxide-releasing aspirin on experimental restenosis. *Proc Natl Acad Sci U S A*. 2002;99:1689–1694.
11. Moons L, Shi C, Ploplis V, Plow E, Haber E, Collen D, Carmeliet P. Reduced transplant arteriosclerosis in plasminogen-deficient mice. *J Clin Invest*. 1998;102:1788–1797.
12. Siegel RJ, Ariani M, Fishbein MC, Chae JS, Park JC, Maurer G, Forrester JS. Histopathologic validation of angioscopy and intravascular ultrasound. *Circulation*. 1991;84:109–117.
13. Kimura T, Kaburagi S, Tamura T, Yokoi H, Nakagawa Y, Yokoi H, Hamasaki N, Nosaka H, Nobuyoshi M, Mintz GS, Popma JJ, Leon MB. Remodeling of human coronary arteries undergoing coronary angioplasty or atherectomy. *Circulation*. 1997;96:475–483.
14. De Geest B, Zhao Z, Collen D, Holvoet P. Effects of adenovirus-mediated human apo A-I gene transfer on neointima formation after endothelial denudation in apo E-deficient mice. *Circulation*. 1997;96:4349–4356.
15. Zhu B, Kuhel DG, Witte DP, Hui DY. Apolipoprotein E inhibits neointimal hyperplasia after arterial injury in mice. *Am J Pathol*. 2000;157:1839–1848.
16. Oguchi S, Dimayuga P, Zhu J, Chyu KY, Yano J, Shah PK, Nilsson J, Cercek B. Monoclonal antibody against vascular cell adhesion molecule-1 inhibits neointimal formation after periadventitial carotid artery injury in genetically hypercholesterolemic mice. *Arterioscler Thromb Vasc Biol*. 2000;20:1729–1736.
17. Chen Z, Fukutomi T, Zago AC, Ehlers R, Detmers PA, Wright SD, Rogers C, Simon DI. Simvastatin reduces neointimal thickening in low-density lipoprotein receptor-deficient mice after experimental angioplasty without changing plasma lipids. *Circulation*. 2002;106:20–23.
18. Perlman H, Maillard L, Krasinski K, Walsh K. Evidence for the rapid onset of apoptosis in medial smooth muscle cells after balloon injury. *Circulation*. 1997;95:981–987.
19. Auge N, Nikolova-Karakashian M, Carpentier S, Parthasarathy S, Negre-Salvayre A, Salvayre R, Merrill AH Jr, Levade T. Role of sphingosine 1-phosphate in the mitogenesis induced by oxidized low density lipoprotein in smooth muscle cells via activation of sphingomyelinase, ceramidase, and sphingosine kinase. *J Biol Chem*. 1999;274:21533–21538.
20. Cybulsky MI, Gimbrone MA Jr. Endothelial expression of a mononuclear leukocyte adhesion molecule during atherogenesis. *Science*. 1991;251:788–791.
21. Penn MS, Cui MZ, Winokur AL, Bethea J, Hamilton TA, DiCorleto PE, Chisolm GM. Smooth muscle cell surface tissue factor pathway activation by oxidized low-density lipoprotein requires cellular lipid peroxidation. *Blood*. 2000;96:3056–3063.
22. Sata M, Walsh K. Oxidized LDL activates fas-mediated endothelial cell apoptosis. *J Clin Invest*. 1998;102:1682–1689.
23. Steinberg D. Atherogenesis in perspective: hypercholesterolemia and inflammation as partners in crime. *Nat Med*. 2002;8:1211–1217.

Stroke
Journal of the American Heart Association

Increased Balloon-Induced Inflammation, Proliferation, and Neointima Formation in Apolipoprotein E (*ApoE*) Knockout Mice

Christian M. Matter, Liming Ma, Tobias von Lukowicz, Patricia Meier, Christine Lohmann, Dongming Zhang, Ülkan Kilic, Eugen Hofmann, Suk-Woo Ha, Martin Hersberger, Dirk M. Hermann and Thomas F. Lüscher

Stroke. 2006;37:2625-2632; originally published online August 31, 2006;
doi: 10.1161/01.STR.0000241068.50156.82
Stroke is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231

Print ISSN: 0039-2499. Online ISSN: 1524-4628